PEARSON, MELAS, AND PS

Pearson marrow pancreas syndrome in patients suspected to have Diamond-Blackfan anemia

Katelyn E. Gagne,1 Roxanne Ghazvinian,2 Daniel Yuan,2 Rebecca L. Zon,1 Kelsie Storm,3 Magdalena Mazur-Popinska,4 Laura Andolina,9 Halina Bubala,6 Sydonia Golebiowska,7 Meghan A. Higman,4 Krzysztof Kalwak,4 Peter Kurre,3 Michal Matysiak,7 Edyta Niewiadomska,7 Salley Pels,8 Mary Jane Petruzzi,5 Aneta Pobudejska-Pieniazek,6 Tomasz Szczepanski,8 Mark D. Fleming,9 Hanna T. Gazda,2,10,11 and Suneet Agarwal1,10,12

Division of Hematology/Oncology, Stem Cell Program, and 2Division of Genetics and Genomics, Manton Center for Orphan Disease Research, Boston Children’s Hospital, Boston, MA; 3Division of Hematology, Oncology and Transplantation, Wroclaw Medical University, Wroclaw, Poland; 4Department of Pediatric Hematology, Oncology and Transplantation, Wroclaw Medical University, Wroclaw, Poland; 5Women and Children’s Hospital of Buffalo, Buffalo, NY; 6Department of Pediatric Hematology/Oncology, Medical University of Silesia, Zabrze, Poland; 7Department of Pediatric Hematology/Oncology, Medical University of Warsaw, Poland; 8Pediatrics, Yale University School of Medicine, New Haven, CT; 9Department of Pathology, Boston Children’s Hospital, Boston, MA; 10Harvard Medical School, Boston, MA; 11Broad Institute, Cambridge, MA; and 12Harvard Stem Cell Institute, Cambridge, MA

Key Points

• PS can be overlooked in the differential diagnosis of children with severe congenital anemia.
• mtDNA deletion testing should be included in the genetic evaluation of patients with congenital anemia of unclear etiology.

Introduction

In 1979, Pearson and colleagues described 4 patients with severe anemia characterized by sideroblasts and vacuolization of marrow precursors, and pancreatic dysfunction.1 Pearson marrow pancreas syndrome (PS) is caused by large deletions in mitochondrial DNA (mtDNA), which accounts for the metabolic acidosis and variable tissue dysfunction in patients.2,3 Deleted mtDNA in patients’ cells exists in varying proportions relative to normal mtDNA, a mixture termed heteroplasmy.4 Changes in heteroplasmy are thought to underlie differences in disease manifestations and evolution in patients,5 including spontaneous hematologic improvement. The incidence of PS is unknown, with only ~100 patients described in the literature since Pearson’s original report.6

Diamond-Blackfan anemia (DBA) is characterized by severe hypoproliferative anemia with erythroblastopenia,7 and variously associated congenital malformations, growth retardation, and elevations in erythrocyte adenosine deaminase and/or hemoglobin F levels.8,9 In 50% to 60% of DBA patients, mutations in ribosomal protein (RP) genes or GATA1 are found, with a high frequency of sporadic cases because of de novo mutations.10-12 The majority of patients respond to an initial course of corticosteroid (CS) therapy, and ~20% of all patients enter a steroid- and transfusion-free “remission” by adulthood.7,13,14 DBA is more frequently occurring than PS, with an estimated incidence of 1-2/100 000 and ~1000 cases in the literature.12,15,16

DBA and PS share important features including early onset of severe anemia, variable nonhematologic manifestations, sporadic genetic inheritance, and episodes of spontaneous hematologic improvement. Because of these features and the rarity of PS relative to DBA, we hypothesized that some patients in whom the leading clinical diagnosis is DBA actually have PS. To test this hypothesis, we retrospectively tested DNA samples from patients enrolled in a DBA genetics research study. Prior studies from this DBA cohort have yielded the novel identification or confirmation of mutations and deletions in RP genes or GATA117-19 in 175 of 362

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There is an Inside Blood Commentary on this article in this issue.

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samples (48%), a proportion similar to that found in other DBA registries.11

Study design

Patient material

Biological samples were procured under protocols approved by the Institutional Review Board at Boston Children’s Hospital and after written informed consent in accordance with the Declaration of Helsinki. The protocol and patient cohort has been described previously.17,18

Long-range polymerase chain reaction (PCR) and deletion mapping

Peripheral blood genomic DNA (50 ng) was amplified using primers 5328F (5‘-CCATCATAGCCACCATCCCTCC-3’) and humitoDloopR (5‘-CTTATGACCCCTGAAATAGGAACC-3’) using iProof HF Master Mix (Bio-Rad). Deletion junctions were mapped using PCR and Sanger sequencing, and nucleotide positions assigned per the revised Cambridge Reference Sequence of human mtDNA (GenBank NC_12920).

Southern blot

Genomic DNA (1 μg) was digested with BbvCI, ClaI, or NheI (New England Biolabs), separated on a 0.6% agarose gel and transferred to nylon membrane. Hybridization of the 32P-labeled probe shown in Figure 1B was performed using Rapid-Hyb buffer (GE Healthcare).

Results and discussion

We used a long PCR strategy to screen DNA samples from the DBA cohort, by amplifying an 11.2-kb region of mtDNA where pathological deletions are found.5 We detected large mtDNA deletions in 8 of 173 genetically uncharacterized samples (4.6%; Figure 1A-B), but in none of 152 patients with known DBA-associated mutations. The detected mtDNA deletions ranged in size from 2.3 kb to 7.0 kb, 5 of which were novel, and 3 of which were previously described, including the 4977-bp “common deletion”20 (Figure 1A, Table 1, and supplemental Table 1 available on the Blood Web site). Southern blot analysis using BbvCI, whose unique site is lost in all deletions, revealed that the deleted mtDNA

Figure 1. mtDNA analysis identifies 8 patients with PS in a cohort of DBA patients. (A) Long PCR, showing the preferential amplification of deleted mtDNA fragments from 8 patients in the DBA cohort, relative to amplification of wild-type (WT) mtDNA. (B) Map of the human mitochondrial genome, with genes (blue), transfer RNAs (tRNAs; yellow), ribosomal RNAs (rRNAs; red), and origins of DNA replication for heavy (H) and light (L) strands indicated. The extent of mtDNA deletions and corresponding patient numbers are shown by the black lines. Purple arrows represent location of the long PCR primers. Southern blot probe used in panels C and D is indicated in green. (C) Southern blot analysis of peripheral blood DNA from patients and normal controls, cut with BbvCI. WT mtDNA shows the expected, 16.5-kb linearized fragment. Deleted mtDNA (∆mtDNA) remains uncut and migrates as circular monomeric and oligomeric species. The lanes shown are from the same blot, rearranged to match the order of patients in the text. Sufficient peripheral blood DNA was not available from patients 2 and 5. (D) Southern blot analysis of peripheral blood DNA from patients and normal controls cut with NheI or ClaI to linearize all species of mtDNA. The proportion of deleted mtDNA (∆mtDNA [%]; ie, heteroplasmy) is shown for each sample. The lanes shown are from 3 different blots, arranged to match the order of patients in the text. Sufficient peripheral blood DNA was not available from patient 5. (E) Bone marrow evaluation of patient 4, showing classic features of PS. Wright-Giemsa stain of bone marrow showing mild hypocellularity and vacuoles in precursors (left; ×40 objective). Magnified image of vacuoles (arrows) in myeloid precursors (center; ×100 oil-immersion objective). Prussian blue stain (for iron) of bone marrow aspirate, showing a ringed sideroblast (arrow) (right; ×100 oil-immersion objective).
Table 1. Clinical and molecular characteristics of 8 patients with PS in the DBA cohort

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<td>—</td>
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ANC, absolute neutrophil count; CMV, cytomegalovirus; Δ, deletion; Dx, diagnosis; Epot, erythropoietin; GCSF, granulocyte colony stimulating factor; HSCT, hematopoietic stem cell transplantation; MCV, mean corpuscular volume; m.o., months old; N.a., information not available; RS, ringed sideroblasts; y.o., years old.

*CS trial planned, but PS diagnosis made prior to intervention.

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Authorship


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Correspondence: Suneet Agarwal, 3 Blackfan Circle, CLS 3002, Boston Children’s Hospital, Boston, MA 02115; e-mail: suneet.agarwal@childrens.harvard.edu; and Hanna T. Gazda, 3 Blackfan Circle, CLS 15023, Boston Children’s Hospital, Boston, MA 02115; e-mail: hanna.gazda@childrens.harvard.edu.

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